BIOFERMEC

KM – Green Yeast Project

Part 1

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Work Proposal

WORK PROPOSAL

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PART 1

CUSTOMER: GREEN YEAST CANADA INC. – LEVURE VERTE CANADA INC.

SUBCONTRACTOR: TECHNOLOGIES BIOFERMEC INC.

1.0 Objectives of project

1.1 Main objective

The main goal of the work to be performed is to produce in the shortest time possible on a medium of whey permeate, the equivalent of 50 kilos of two KM yeast products, in a 50:50 ratio:

- Active yeast
- Autolyzed/hydrolyzed inactive yeast

1.2 Specific objectives

- To better understand the growth parameters of the strain *K. marxianus* GY
- To define and establish the parameters for control and QC during fermentation
- To optimize the growth medium for *K.marxianus* GY by using nutritional additives
- To optimize the production of inoculum
- To define and validate the fed-batch mode of propagation11
- To produce and dehydrate the three types of yeast products
- To perform analysis and evaluation of the three yeast products

2.0 Criteria

The finished products must be representative of the future products which will be generated by the novel continuous process owned by Green Yeast.

3.0 Overall procedure

In order to respect the above-mentioned criteria, we must quickly reproduce the Green Yeast fermentation technology.

3.1 Inoculum

In the continuous process, the properties of the initial inoculum and maintaining it are the essential factors in order to repeatedly obtain high cellular biomass yields, those being the inescapable conditions for the success of the technology.

We will therefore have to put into collection pure and active yeast strains, and propagate them in the best growth media in order to finally generate an always optimal inoculum.

This will make up the initial steps.

3.2 Analytical work

Total solids :

- ^{O} Brix using refractometer
- % total solids using oven drying, 12 h at 105^{o}C.

Acidity:

- pH
- titratable acidity as lactic acid

Cells:

- cellular biomass by centrifugal collecting and oven drying
- direct count using microscope
    - total cells per ml
    - dead cells per ml
    - budding cells per ml

- CFU counts on PDA agar
- Optical density in spectrophotometer

Dissolved Oxygen:

- DO using titration (1 ~ 10 ppm)

Contamination:

- Counting of coliforms on VRBA
- Counting of total bacteria on PCA

Crude Protein, crude lipids, lactose, ash: external commercial labotatory.

3.3 Culture medium

The inoculum will be produced on a YME and YKF propagation media, both with 1,2% of added whey permeate solids

One ml of frozen yeast sample from the collection will be reactivated by incubating for 24 hrs at $30^{o}C$ in a tube containing 5 ml of sterile YME medium

Tube contents will then be used to inoculate (in duplicate) 250ml Erlenmeyer flasks containing 100 ml of YME or YFK medium with 1,2% added whey permeate solids. Flasks will be incubated with agitation at $35^{o}C$.

Growth parameters (D.O., pH, live cells, dead cells, buds, CFU counts on PDA) will be monitored in order to determine the optimal length of fermentation.

The resulting optimal inoculum wll be used to study in 150 ml flasks, the various nutrients and also to quantify the acid-producing rate. We will then be able to measure the amounts of Ammonium Hydroxide necessary to maintain the pH at 4.6. This step will further help us determine the amounts of either Ammonium Sulphate or Urea to add to the culture medium.

It will also be possible to evaluate the possible advantages of adding niacin and d-pantothenic acid as micronutrients.

The optimal growth curve will be traced as function of the best cellular biomass yields. From this curve, the shortest generation time will be determined and will be used as initial point for feeding sterile medium.

This generation time will also allow us to determine the amount of medium to feed per hour in order to maximize yields.

In conformity with the model which we will have designed, fedbatch trials will be performed on 150 ml volumes in 250 ml flasks stirred at 35^{o}C.

Since we can mathematically expect a 100 % yield of volume per day, we will try additions of 6 ml per hour (150 ml over 24 h) per flask. We will also try addition levels of 4 and 7 ml in order to calculate the effect on yield.

3.4 Production

The first production trials will be performed on 25 liter volumes of medium which will correspond to approx. 1.5 kg of cellular biomass per day. The crude production expected would be 25 liters per day or an addition feeding rate of 18 ml per minute.

This liquid will be bottled in 10 L containers and kept in the cold in order to let the yeast cells settle and to later separate the settled concentrated phase from the light upper phase.

As soon as we will have mastered production during three (3) days, we will initiate 100-liter production runs, which should generate 5~6 kg of dry cellular biomass per day.

4. Activities

Activities are explained in detail in Annex 1. A coordination meeting will take place every 2 weeks at BioFermec's offices in order to discuss the status of the work.

5. Costs of the work

As detailed in Annex 1 we must work with budgetary forecasts. If things go well, BioFermec will be able to stay closely on budget. There are however several unknowns which we cannot anticipate at the moment. Rest assured that we will work very closely with you and that you will be notified in realtime as work progresses.

The following costs have to be taken into account:

Analytical testing, and rental of equipments for drying of products.

BioFermec budgetary costs:	16,800 $

Other budgetary costs to validate and to be paid directly by the customer:

- Equipments property of customer	6,350 $
- Chemical analysis	1,500 $
- Rental of drying equipments	2,350 $
TOTAL	27,000 $

6. Deliverables

Conditional to receiving the equipments to be provided by the customer and listed in Annex 2, BioFermec undertakes to deliver:

- Monthly summary report

- Final report at the end of project, scheduled for late July.

7. Knowledge passed on to customer

Studies performed, and results obtained and protocols developed in the course of the project will be the exclusive property of the customer. Any request for grants or tax credits remain the customer's responsibility.

8. Payment conditions

BioFermec will invoice its hours at the beginning of each month and the invoice will be payable within 10 working days.

9. Intellectual property

All information and discoveries which may result from the above work are the exclusive property of the customer.

Signed on _____

M. Daniel Trudeau, President and CEO

Green Yeast Canada Inc.

Marien Lavoie, President

BioFermec Technologies Inc.